The Development of the Semilunar Valves in the Human Heart

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The development of human semilunar valves was studied in a range of specimens including embryos before the appearance of cellular semilunar valves at stage 17 and hearts after the attainment of mature fibroelastic valvular structure, which occurs around the time of birth. The valves develop by modification of endocardial cushion material at the downstream end of the cardiac tube and appear to grow at the margins, probably by cellular proliferation into a stagnant zone caused by boundary layer separation of blood flow. The flat endothelium on the ventricular aspect and the cuboidal endothelium on the arterial aspect of the valves correlate, respectively, with expected high and low shear forces produced by surface blood flow. Development of an aorta and pulmonary trunk with tricuspid semilunar valves appears to be contingent on the appearance of separate entwined ventricular ejection streams. The later fibroelastic phases of semilunar valve development show progressive increase in elastic and collagenous fibers, at sites which appear to be subjected, respectively, to fluctuating and static tensions (Am J Pathol 74:331–344, 1974).

PREVIOUS INVESTIGATORS have described the early development [1,2] and mature structure [3] of human semilunar valves. This study traces the morphologic changes in the semilunar valves from their first appearance to the acquisition of adult form. An attempt is made to explain the sequential changes in valvulogenesis as effects of hemodynamic forces. An understanding of the processes of normal development may facilitate the elucidation of the pathogenesis of malformations of the semilunar valves and of the responses of the intimal tissues of the cardiovascular tree to abnormal conditions.

Materials and Methods

Observations were made on over 150 human specimens from 3 weeks gestation to 10 years of age. Serially sectioned embryos from the Carnegie Embryological Collection including stages 10 (22 days of gestation; 2 mm length) to 23 (56 days of gestation; 30 mm crown-rump length,) [4–9] were studied. Embryos cut in sagittal, frontal and transverse planes were reviewed for stages 14 to 23, in the transverse and frontal planes for stages 12 and 13, and in the transverse plane for stages 10 and 11. Most embryos were stained with alcohol cochineal (AC) or hematoxylin and eosin (H & E).

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Forty embryos and fetuses were obtained from the Johns Hopkins Hospital Obstetrical Service and 30 specimens from older patients from the Department of Pathology. Seven specimens of varying crown-rump length (CRL): 10 mm (stage 16), 15 mm (stage 18), 20 mm (stage 19), 18 mm (stage 20), 21 mm (stage 21), 34 mm, 36 mm and 45 mm were serially sectioned at 10 μ and selected sections stained with either H & E, Verhoeff-van Gieson (VVG), Masson's trichrome or alcian blue. Normal aortic and pulmonary valves of 33 fetuses (50 to 200 mm CRL), 17 immature, premature and term newborn infants (800 to 2200 g), and 13 infants and children (6 weeks to 10 years of age) were examined with histologic sections, 4 μ thick, stained with H & E, VVG or alcian blue.

Topographic relationships of the semilunar valves and great vessels were determined in three staged embryos by reconstructions made from tracings of serial sections. Tissue sections were projected at a magnification of 62 × and details of the valves, outflow tracts, and great vessels were recorded on transparent paper. Anatomical reconstruction was made by stacking sections using the vertebral column as a reference point in the sagittal plane. The course of blood flow was approximated by plotting the mid points of the ventricular cavities, outflow tracts and great vessels in each cross section.

Results

The developing heart is a tube with a single lumen and no evidence of semilunar valves at stage 14 (Figure 1). In stage 15 an H-shaped outflow channel with four mounds of endocardial cushion tissue is usually present. The mounds, which extend only to the downstream end of the myocardial mantle, are covered by a single endothelial layer and consist of stellate and spindle-shaped cells in a loose matrix. By stage 16 (9 mm CRL) the aortic and pulmonary outflow tracts are distinctly separate (Figure 2). The downstream ends of the intimal mounds acquire a valve-like apperance by stage 17 (Figures 3 and 4). The endocardial cells on the ventricular aspect of the valve leaflet are flat in comparison to the cuboidal configuration on the arterial side (Figure 4). Valve sinuses appear as shallow depressions between the arterial wall and the developing valves. At stage 18 (15 mm CRL) the coronary arterial system appears.

By stage 21 (23 mm CRL) the valve leaflets are short and thick and fill most of the deepening sinuses (Figures 5 and 6). There is a progressive increase in cellularity of the leaflets. The cells in the valve are spindle-shaped and oriented with their long axis perpendicular to the axis of the leaflet (Figure 6). At stage 23 (30 mm CRL) the leaflets have become thinner and more delicate. From this stage on there is further refinement in leaflet shape with progressive elongation, thinning and tapering of the edges. Alcian blue-positive ground substance is prominent in the early embryonic valve but gradually diminishes until little appears in the fetal valve over 150 mm CRL.

Wavy, slender and unorganized elastic fibers are present in the media

of the great vessels at stage 17, but distinct lamellae do not appear until 35 mm CRL, at which time fine collagenous fibrils are visible in the leaflet attachment at the annulus fibrosa and the adventitia of the great vessels. The 45 mm CRL fetus has fine collagenous fibrils in the base of the valve commissures. At 60 mm CRL, the valve leaflet loses its homogeneous cellular character. Collagenous fibrils are visible in the most proximal part of the valve leaflets and in the ventricular subendocardium. At 90 mm CRL there are collagenous strands in the proximal one-third of the leaflet, the media of the great vessels and valve commissures. A loose myxomatous zone is present below the line of closure on the ventricular side of the leaflet between the collagenous core and endocardium.

A distinctive subendocardium appears at 100 mm CRL. Delicate elastic fibers continue from the subendocardium of the ventricles to the subendocardium on the ventricular side of the semilunar valve leaflets. Collagenous fibrils are present up to the line of leaflet contact during valve closure (Figure 7). By 125 mm CRL the proximal three-fourths of the leaflet is filled with denser collagenous tissue. There is also a concommitant increase in collagenous material in the media of the great vessels from 60 mm CRL to 125 mm CRL.

In the 150 mm fetus the semilunar valves begin to adopt a mature structure. Dense collagenous tissue is sharply demarcated into a longitudinal central core and a loose connective tissue layer is present on the ventricular side of the leaflet from its base to the line of closure. As the central collagenous plate develops there is gradual extension of the subendocardial elastic layers on both the ventricular and arterial aspects of the leaflet, until elastic fibers extend entirely around the leaflet tip in the newborn infant. At birth the leaflets have a mature structure with definitive endocardial and subendocardial layers, a loose connective tissue zone and collagenous core.

Capillaries or other blood vessels are not identified in the embryonic and fetal valve leaflets, commissures or annulus, although small capillaries were seen in the base of leaflets in occasional adult specimens. No muscle cells or nerve fibers were observed in the developing leaflets.

There are not apparent morphologic differences between the aorta and pulmonary trunk up to the time of birth. A general increase in the number of lamellae in the great vessels occurs up to about 150 mm CRL. Thereafter, the number of lamellar rings remains relatively constant but there is a marked increase in the thickness of individual lamellae. After birth there are consistent changes including increased thickness of the aorta relative to the pulmonary trunk and the compact arrangement

of parallel elastic lamellae is sparser and incomplete in the latter. Elastic lamellae do not develop in the attachment of the valves to the vessel wall. This region consists almost entirely of collagenous fibrils (Figure 8).

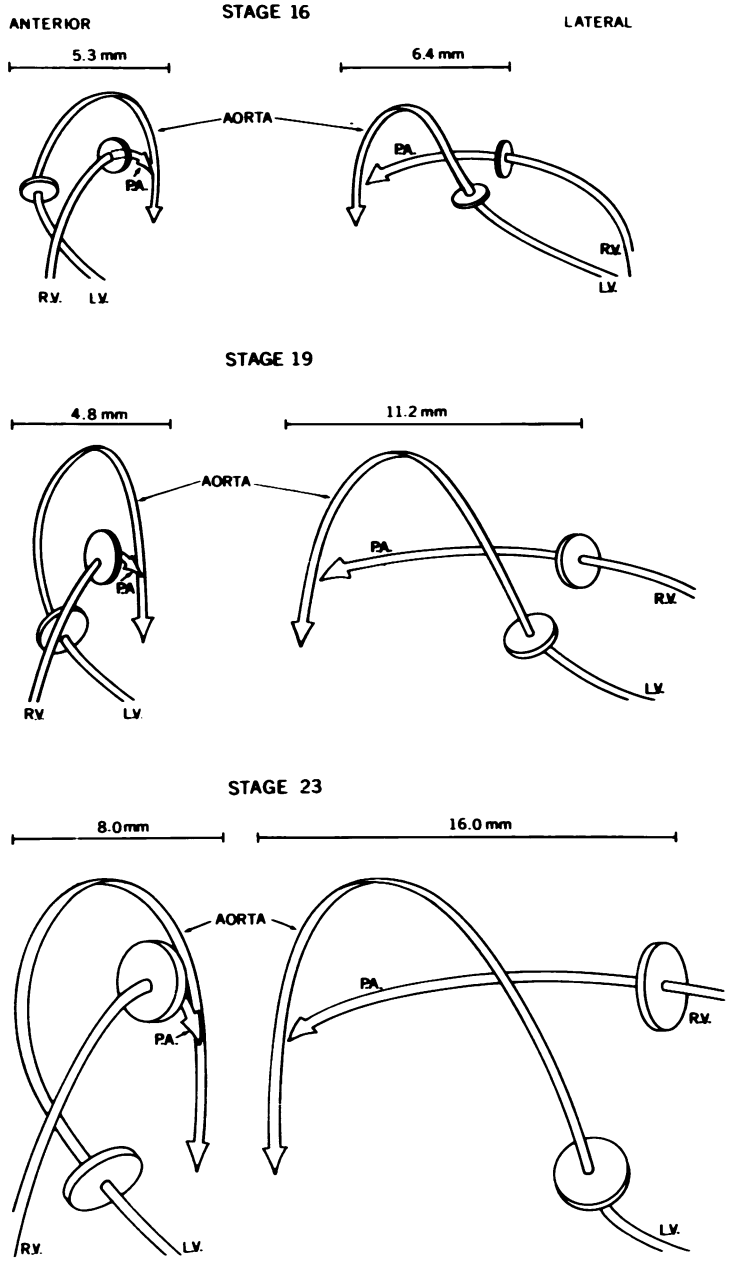
Prior to birth the histologic structure of the two semilunar valves is identical (Figure 9). During subsequent development there is a relative increase in collagenous and elastic fibers in the aortic valve leaflets (Figure 10) as compared to the pulmonary valve.

The changing spatial relationship between the ventricles, outflow tracts, semilunar valves and the great vessels is shown in Text-figure 1. At stage 16 two outflow paths can be identified although anatomic separation may not be complete. The valves are positioned lateral to each other, with the pulmonary valve slightly superior and anterior to the aortic valve. At stage 19 the pulmonary valve becomes still more anterior and superior. The aortic valve is medial and the valves are in nearly the same sagittal plane. At stage 23 there is little change from the previous stages and the entwined relationship of the right and left ventricular outflow paths, the relative position of the semilunar valves, and the course of great vessels are essentially those observed in stage 19.

Discussion

Early semilunar valve development is synchronized with the transformation of the single lumen cardiac tube into a heart with two separate ventricles, outflow tracts and great vessels. The early heart tube (stages 10 to 12) consists of outer myocardial and inner endocardial layers separated by acellular cardiac jelly.[10] The jelly is an acid mucopolysaccharide [11] produced by the myocardial cells [12] and occurs only in parts of the cardiac tube invested by myocardium. The gradual conversion of cardiac jelly into endocardial cushion tissue by infiltration and proliferation of cells, which appear to be derived from the endocardium, begins at stage 12.

At stage 14 the outflow tract is still a single tube (Figure 1) with its endocardium in continuity with the endothelium of the aortic sac. Unlike the endocardium of the cardiac tube, which is separated from the myocardium by cardiac jelly, the endothelium of the aortic sac is in intimate contact with surrounding mesenchymal cells. Fusion of the endothelium to the mesenchyme appears to prevent progression of the cardiac jelly beyond the downstream end of the myocardial tube. The term truncus arteriosus has not been used here because of confusion with the malformation complex truncus arteriosus.[13] There is



TEXT-FIG 1—Diagram of the axes of the right and left ventricular outflow tracts, pulmonary trunk, and aorta (*ribbons*) and pulmonary and aortic valves (*discs*) based on reconstructions of serial sections of embroys of stages 16, 19 and 23. Shown in anterior-posterior and right lateral projections.

no common vessel downstream from the limits of the myocardial mantle [14] other than the aortic sac, which has disappeared by stage 16 (Figure 2).

The development of separate ventricular chambers, which begins at stage 14, may be responsible for a major revision of the heart through formation of separated ejection streams.[2] The direction of the outflow stream from the right ventricle is toward the left sixth arterial arch while left ventricular ejection is toward the fourth arterial arches. The mesenchyme downstream from the cardiac tube, which invests these preferred arterial channels of flow, represents the so-called aortic-pulmonary septum.[1] Rather than a distinct structure which actively divides the aortic sac into two great vessels, the "septum" appears to be mesenchyme filling in the space between the two paths of blood flow (Figure 2).

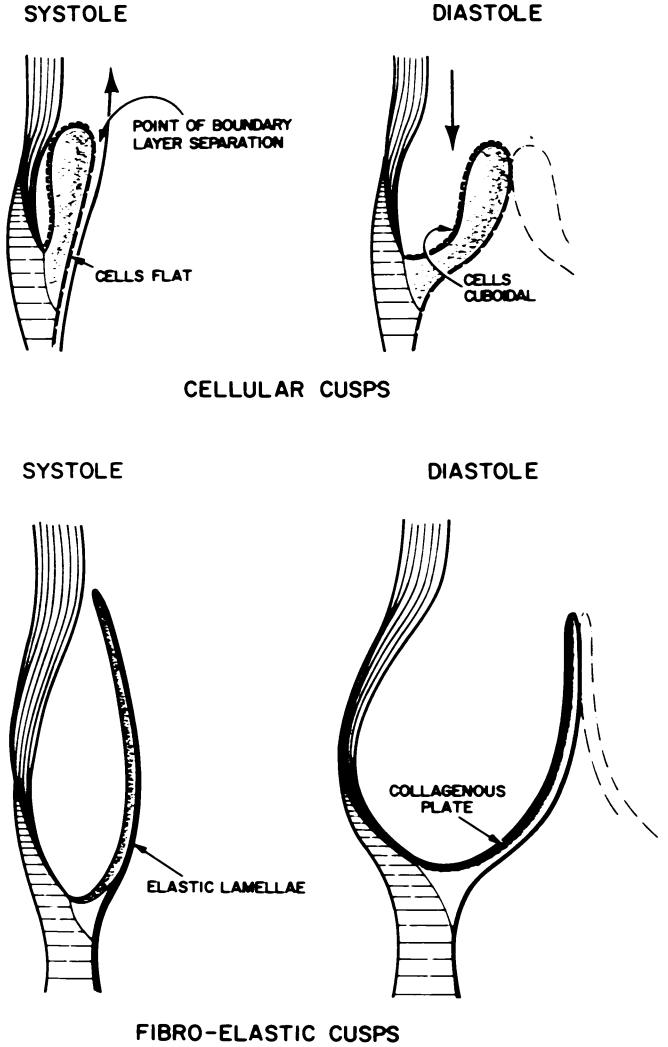
The two entwined outflow streams of the early ventricles appear to remold the gelatinous cardiac jelly so that the cylindrical tube of the single outflow tract of stage 14 is converted to an elliptical H-shaped lumen by stage 16. The H-shaped cross-section represents the accumulation of the endocardial cushion material into four mounds (two large and two small) and is probably due to the development of two separate outflow streams and also the compression of the downstream end of the cardiac tube between the atria and the anterior body wall. The H-shaped lumen and four mounds may reflect an equilibrium configuration of least tension for the semisolid cushion material confined between the elliptical myocardial wall and the two streams of ventricular outflow. Analagous configurations are found in a number of biologic systems.[15]

With division of the aortic sac into pulmonary trunk and aorta, the mesenchyme surrounding the vessels appears to fuse with the downstream end of the two large mounds of endocardial cushion material. The large cushions are fused and divided so that three approximately equal mounds of cushion material protrude into each arterial lumen. The pulmonary valve is derived from the three mounds in the right ventricular outflow tract and the aortic valve from the three mounds in the left ventricular outflow tract.

The semilunar valve cusps arise by remodeling of the endocardial cushion tissue at the junction of the cardiac tube and the arterial mesenchyme. Cell proliferation and infiltration into the cardiac jelly at the downstream end of the heart tube "fixes" the endocardial mounds at the site of the future valves.

Flattened endothelial cells on the ventricular aspect of the valves

and cuboidal cells on the arterial side were observed consistently from the earliest appearance of valves at stage 16. The shearing effect of the blood column during ventricular ejection may be responsible for flattening of the endothelial cells on the ventricular aspect. The ejection stream flowing over the ventricular surface of the valve cusps would break free at the edge of the leaflet and enter the aortic lumen. The edge of the leaflet would thus be a point of "boundary layer separation" (Text-figure 2) with local turbulence of blood flow and dif-



TEXT-FIG 2—Diagram of the early cellular (*top*) and mature fibroelastic stages (*bottom*) of semilunar valve development.

ferent hemodynamic characteristics from adjacent areas. The absence of significant blood flow parallel to the surface on the arterial side of the valve leaflet, despite the higher lateral pressure, may explain the cuboidal configuration of endothelial cells on that surface.

The early valve cusp is quite cellular, with a mucopolysaccharide matrix but no identifiable connective tissue fibers. The cusps appear to grow by proliferation of cells on the bulbous downstream ends (Text-figure 2). A similar appearance is found in abnormal valve-like structures on the endothelium or endocardium in certain pathologic conditions[16–18] and in experimental valvulogenesis.[19] This pattern of growth appears to be explained by cellular proliferation into a zone of low pressure and low shearing force at the valve tip. Boundary layer separation of the ventricular systolic ejection stream from the valve endocardium at the tip of the cusp could produce a stagnant zone of low pressure and a low shearing force, which are conducive to endocardial proliferation.

Collagenous and elastic fibers appear in the developing valves and increase progressively in amount and in organization. Collagenous fibers are found in anatomic sites exposed to static tension.[20] Elastic fibers are present normally in tissues subjected to fluctuating tension. The arrangement and amounts of these fibers may be taken as sensitive indicators of the type of tension to which the tissue is exposed.[21,22] Elastic lamellae are very prominent on the ventricular aspect of the leaflet, which is exposed to intermittent tension by systole. The arterial side of the leaflet, exposed to the more static tension of diastole, consists primarily of a collagenous plate. At the line of closure the whole thickness of the cusp is composed of the collagenous plate, correlating with the equal tension on both sides of the leaflet at this point.

Semilunar valve leaflet growth is limited by the diameter of the valve orifice. Leaflet growth appears to occur by continued cellular proliferation at the point of boundary layer separation of systolic flow. However, when the leaflet becomes long enough to contact the arterial wall above the sinus of Valsalva during systole, boundary layer separation and cellular proliferation could be expected to stop (Text-figure 2).

The aorta and pulmonary trunk acquire elastic lamellae early in development. There is a subsequent increase in the number of lamellae and the thickness of individual elastic fibers. The number of lamellar units correlates generally with the arterial diameter and presumed degree of mural tension.[23] Up until the time of birth the aorta and pulmonary trunk, like the semilunar valves, are histologically indistinguishable. After birth, the lowered pressure in the pulmonary circulation

is reflected in more delicate valve leaflets and a loose arrangement of elastic lamellae in the pulmonary trunk.

In conclusion, the appearance and formation of semilunar valve cusps appears to be contingent on the separation of the single outflow stream of the early heart tube into two separate ejection streams. The early cellular valve leaflets are prolongations of endocardial cushion material which develop from the cardiac jelly of the early myocardial tube. The valve cusp grows by cellular proliferation, probably induced by boundary layer separation of the blood flow stream. Elastic fibers and collagenous constituents of the mature valve are present in locations and amounts corresponding, respectively, to the fluctuating and static tensions expected in the valve leaflets.

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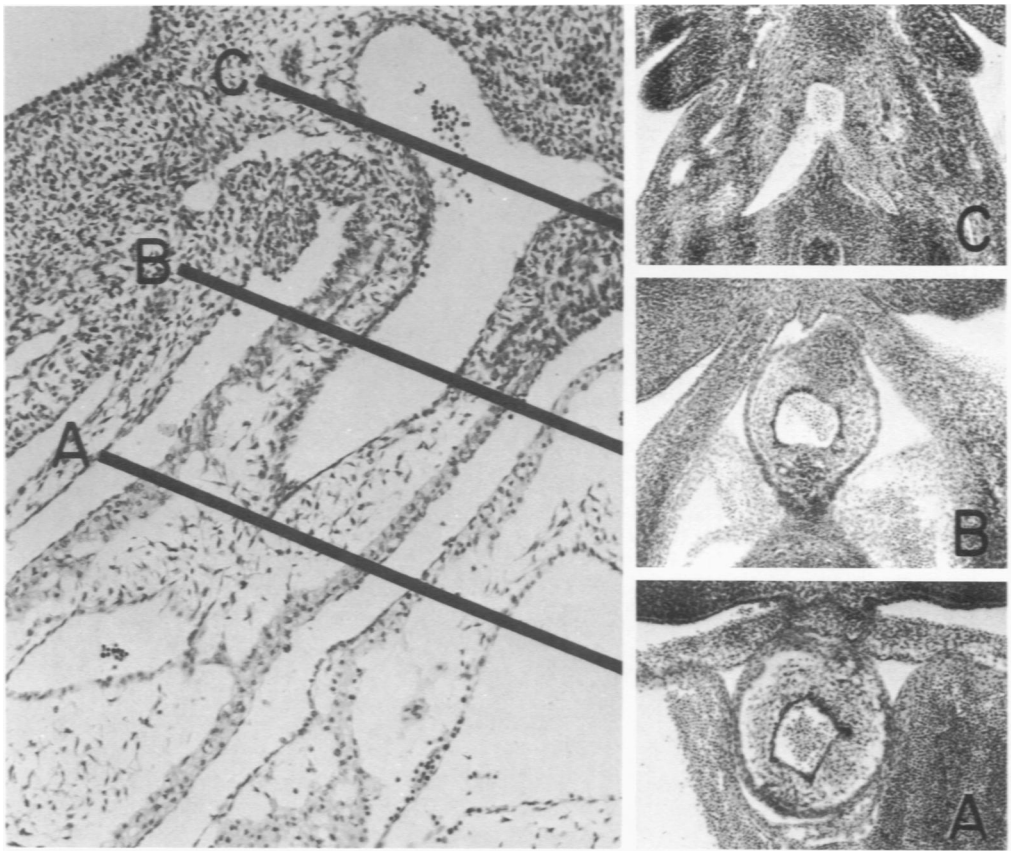
Legends for Figures

Fig 1—On the left is a sagittal section through the downstream end of the cardiac tube of a stage 14 embryo (Carnegie Embryo 3787). The tongue is at the upper left and the atrial cavity at the lower right. On the right are transverse sections through the cardiac tube of an embryo which is also at stage 14 of development (CE 4245, AC, × 65). The lettered transverse levels correspond to the lettered lines on the sagittal section. In *A* and *B* the tongue is at the top and the atrial cavities are at the bottom. Section *A* passes through the single lumen midline outflow tract at a level below where the semilunar valves will develop. Section *B* is at the approximate junction of myocardium with the mesenchyme and is the level at which the semilunar valves will appear. At level *C* the sixth aortic arches arise symmetrically from the midline aortic sac and run posteriorly.

Fig 2—On the left is a sagittal section of a stage 16 embryo (CE 1544, AC, × 115) and on the right are transverse sections of a stage 16 embryo (CE 6507, AC, × 135) both oriented as in Figure 1. At level *A* there is no anatomical separation of the right ventricular outflow tract which is anterior and to the left of the midline from the left ventricular outflow tract which is posterior and to the right of midline. At level *B*, just proximal to the end of the myocardium, there is anatomical separation of the outflow tracts by mesenchymal cells. The aortic valve leaflets are seen at the right in transverse section *B*. At level *C* the pulmonary trunk (*arrows*) lies to the left and the fourth arterial arches arise symmetrically from the aorta.



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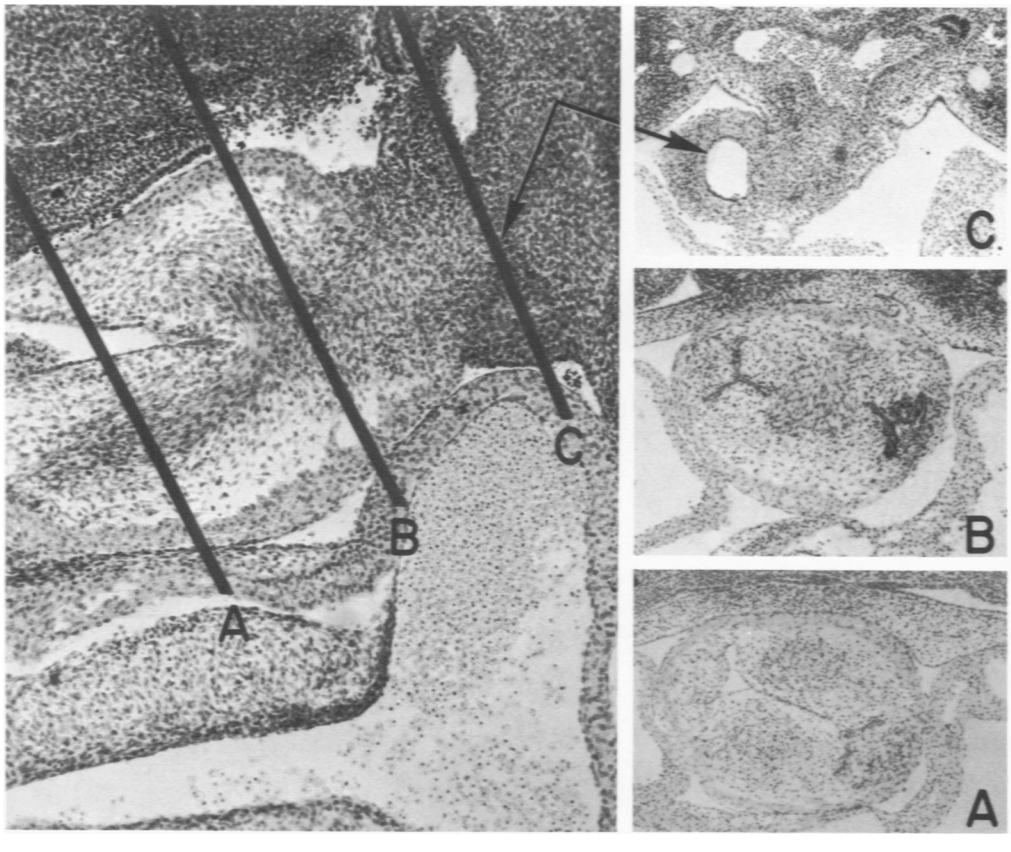

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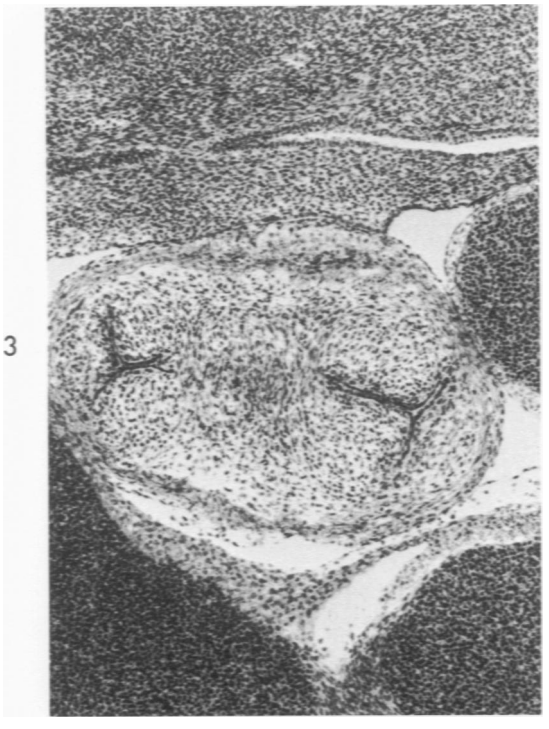
Fig 3—Frontal section of a stage 17 embryo (CE 8998). The cardiac tube lies between the atria and the anterior body wall. Three endocardial mounds form aortic (*right*) and pulmonary (*left*) valves (Mallory azan, × 75).

Fig 4—Sagittal section of a stage 17 embryo (CE 6519) showing aortic valve leaflets with sparse mesenchymal cells. There is cuboidal endothelium on the arterial aspect and flattened endothelium on the ventricular side of the leaflets. The downstream end of the myocardium is at the *arrow* (AC, × 200).

Fig 5—Frontal section of a stage 21 embryo (CE 7864) showing leaflets as bulbous cellular structures largely filling the sinuses (H&E, × 75).

Fig 6—Sagittal section of a stage 21 embryo (CE 5596) showing cellular leaflets. The *arrow* indicates the downstream end of the myocardium (AC, × 200).



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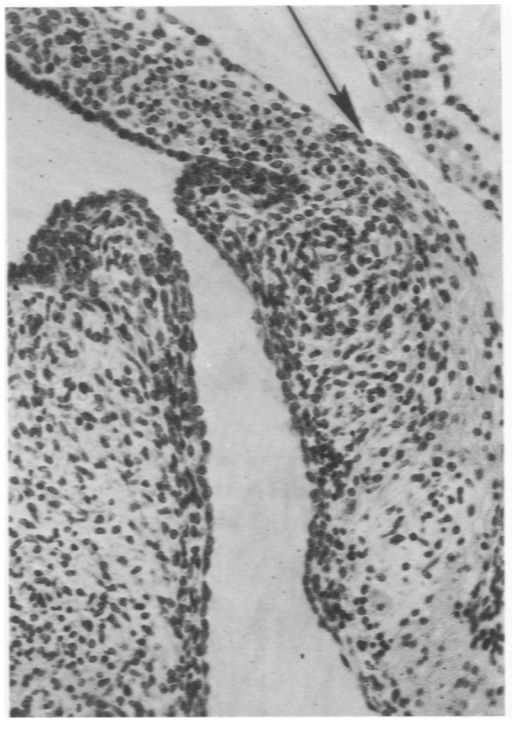

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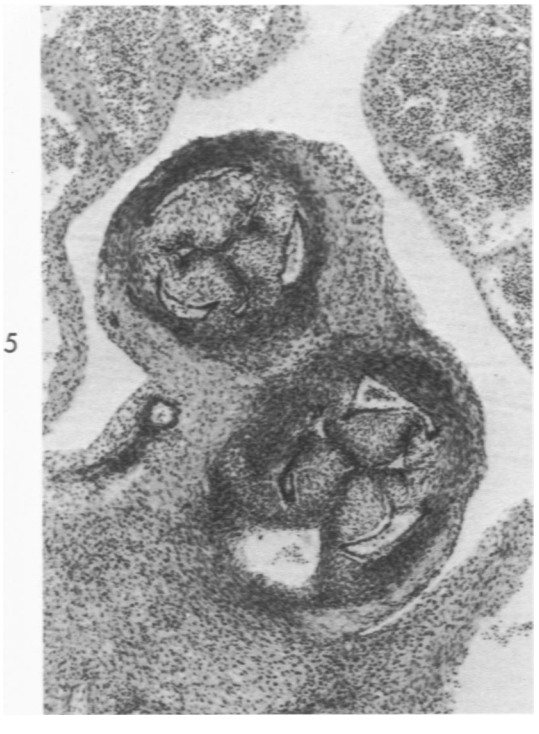

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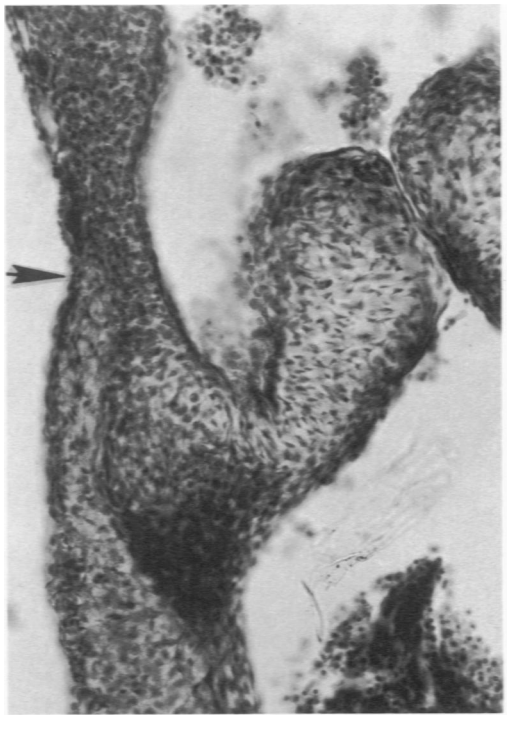

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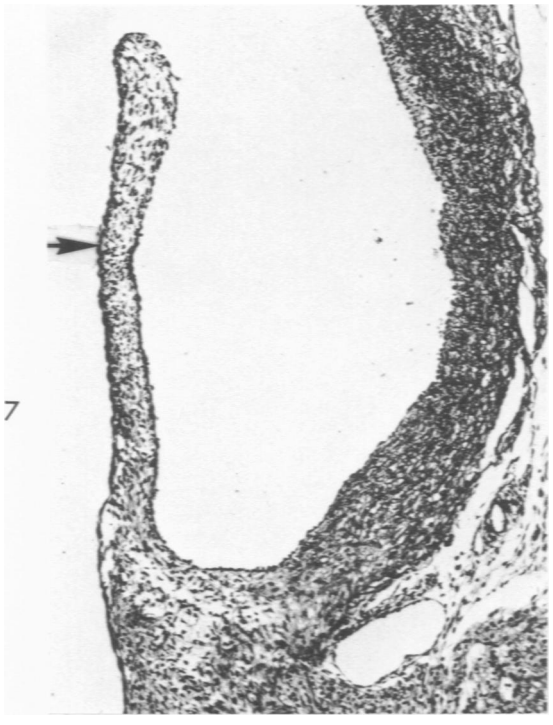

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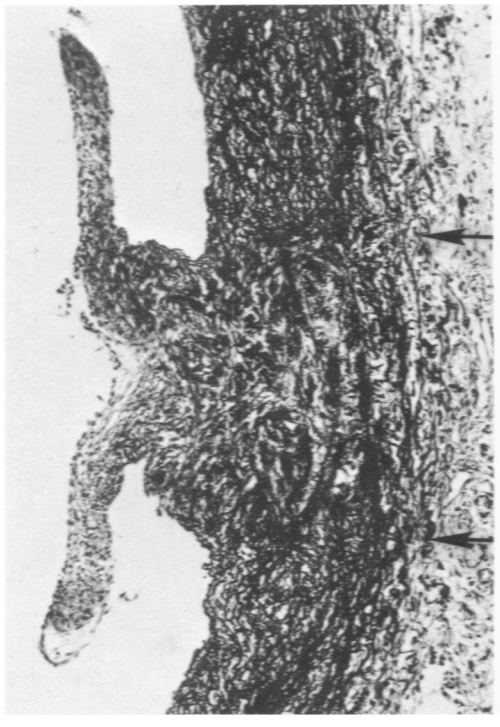

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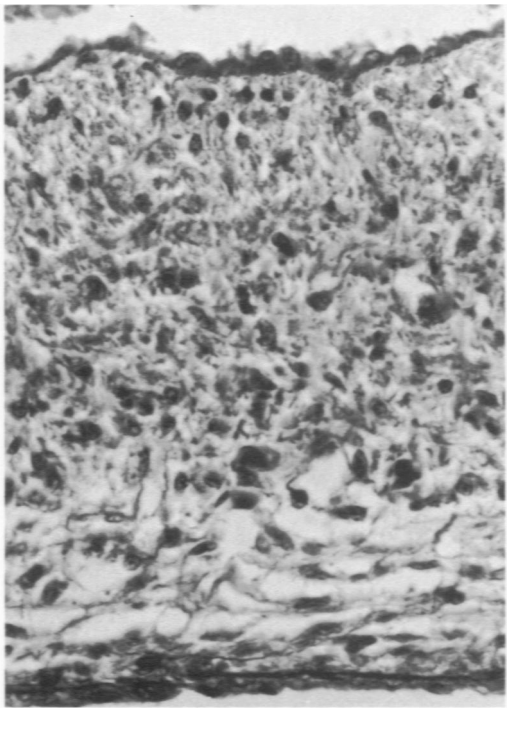

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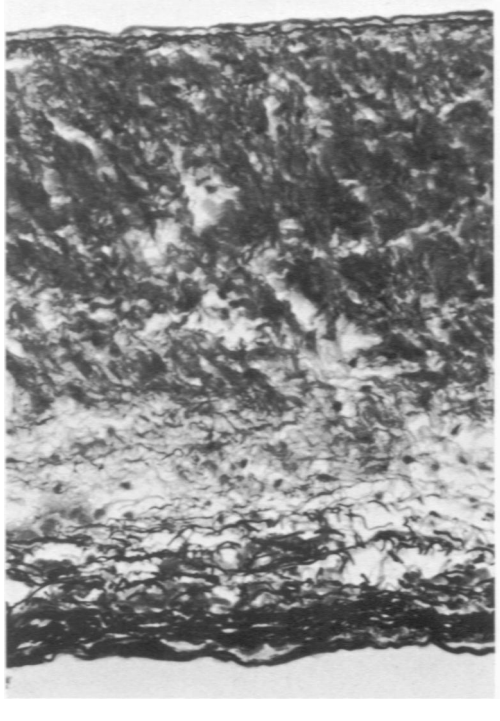

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Fig 7—Longitudinal section through the aortic valve of a 100 mm CRL fetus showing the early fibroelastic stage of valve development. Collagen extends to the line of closure (*arrow*) of the leaflet (VVG, × 90). **Fig 8**—Transverse section through a commissure and leaflets of the pulmonary valve of a 175 mm CRL fetus. There is sharp demarcation between elastic lamellae in the sinuses of the pulmonary artery exposed to fluctuating tensions and the collagen (*between the arrows*) in the commissure which is subject to more static tension (VVG, × 90). **Fig 9**—Cross-section of the left lateral pulmonary leaflet of a 1200-g premature newborn. *Top*, arterial side; *bottom*, ventricular side with a few delicate elastic fibers and a single lamella in the endocardium. The collagenous plate is on the arterial side of the leaflet (VVG, × 430). **Fig 10**—Cross-section through the right lateral aortic leaflet of a 3½-year-old child showing the mature fibroelastic valve. Thick elastic lamellae are present in the subendocardium on the ventricular side of the leaflet at the bottom. The dense collagenous plate is on the arterial side of the leaflet at the top (VVG, × 215).